

Mechanism(s) of action and potency of Hsp90 inhibitor ganetespib in small cell lung carcinoma cells

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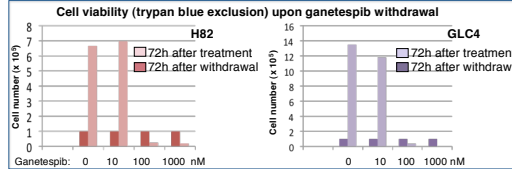
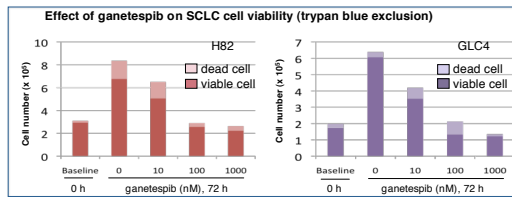
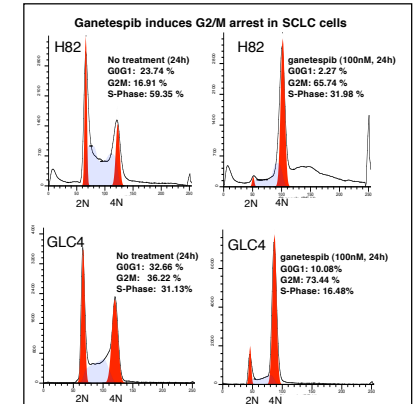
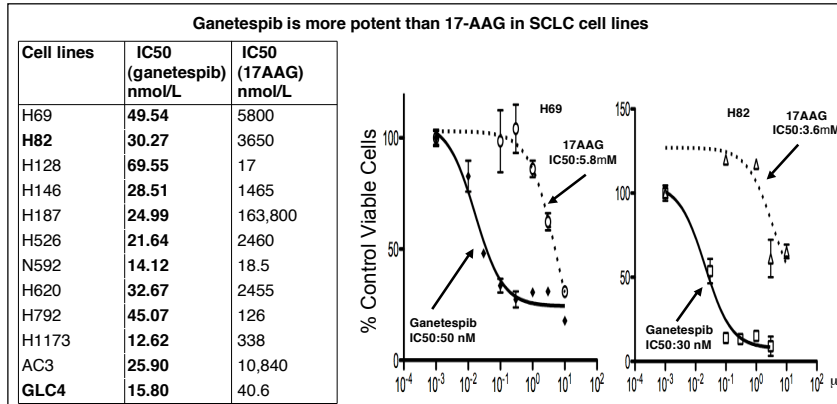
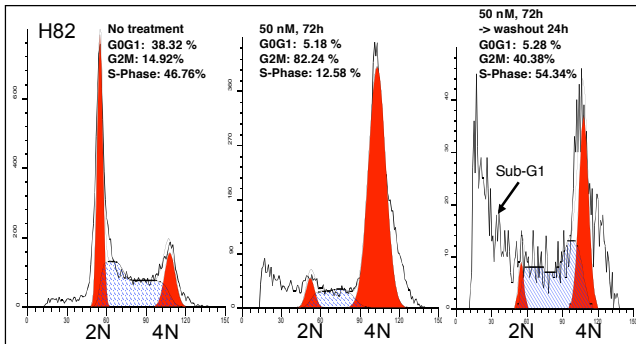
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Abstract

Background: Ganetespib (formerly STA-9090, Synta Pharmaceuticals) is a second generation Hsp90 inhibitor, which is a resorcinol-containing compound with a novel structure that is unrelated to the geldanamycin class of Hsp90 inhibitors. It binds to the ATP-binding domain of Hsp90 and is a potent Hsp90 inhibitor through degradation of Hsp90 client onco-proteins in cancer cells. At low nanomolar concentrations, ganetespib potently inhibits cell proliferation and induces apoptosis in a variety of cancer cell lines including many receptor tyrosine kinase inhibitor- and 17-AAG (geldanamycin class of Hsp90 inhibitor)-resistant cell lines, and in several tumor xenograft models. Based on preclinical activity of other Hsp90 inhibitors, small cell lung cancer appears like a promising tumor target for this class of agents.

Results: Using 12 small cell lung carcinoma (SCLC) cell lines, we demonstrate that ganetespib (IC₅₀: 30.9 ± 16.6 nM) is much more potent than 17-AAG (IC₅₀: 16 ± 47 μM) in MTS assays. In addition, at the concentrations of IC₅₀ or 3-60 times over IC₅₀, ganetespib exhibits cytostatic effect by arresting cells at G2/M on majority of the SCLC cell lines studied so far. Cells survive for as long as 6 days in the presence of ganetespib. Intriguingly, cell viability precipitously drops upon ganetespib withdrawal following 48-72 h treatment with the majority of the survival cells remaining in G2. Sequential ganetespib followed by doxorubicin treatment significantly reduced RB-/- H82 cells viability. The effect of ganetespib and doxorubicin combination on SCLC xenograft cells are currently under investigation.



Ganetespib-induced G2M arrest are predominantly in G2 and irreversible

Cell lines	H82 phospho-H3+ (%)	GLC4 phospho-H3+ (%)
No Tx	2.89	0.42
24hTx (50nM)	6.94	12.38
48h Tx (50nM)	6.01	4.2
72h TX (50nM)	4.26	4.65
Washout 24h after 72h TX (50nM)	1.12	5.55
Washout 48h after 72h TX (50nM)	3.19	3.07
Washout 72h after 72h TX (50nM)	0.42	1.43

Each includes counting 10 random areas of phospho-H3 (Ser10) antibody stained slides.

Effect of ganetespib and doxorubicin combination on H82 cells

	0 h total viable cell (viability)	24 h total viable cell (viability)	48 h total viable cell (viability)
No treatment	5.61*10 ⁴ (100%)	7.90*10 ⁴ (93.3%)	3.08*10 ⁵ (90.2%)
doxorubicin 600nM 24h → ganetespib 50nM 24h	5.61*10 ⁴ (100%)	6.22*10 ⁴ (64.4%)	5.06*10 ⁴ (13.6%)
ganetespib 50nM 24h → doxorubicin 600nM 24h	5.61*10 ⁴ (100%)	2.24*10 ⁴ (23.5%)	2.83*10 ⁴ (11.4%)

Cell viability was assessed by trypan blue exclusion

Conclusion

1. Ganetespib is more potent than 17-AAG in SCLC cells.
2. Ganetespib inhibits RB-negative SCLC cell growth via induction of G2/M arrest and cell death.
3. Ganetespib withdrawal induces precipitous cell death suggesting that ganetespib intermittent treatment strategy might be considered for the ongoing phase II studies in SCLC.
4. Sequential ganetespib → doxorubicin treatments are synergistic in RB-/- H82 SCLC cells.

